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True *Arrhenius* Relationships of Human Lactate Dehydrogenase

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The pyruvate and NADH concentrations and the buffer pH which gave maximal activity with LDH isoenzymes derived from human heart and liver tissue were established for the temperatures 25°C, 30°C, 35°C, 37°C, 40°C, 45°C, and 50°C. The velocities of the LDH isoenzymes using these maximal assay conditions were used to obtain *Arrhenius* plots, i.e. log initial velocity against inverse absolute temperature. The *Arrhenius* plots were linear with both isoenzyme preparations up to 45°C. Between 45°C and 50°C it appeared that this linear relationship no longer held, particularly with the liver tissue. When the activation energies were calculated both isoenzyme preparations exhibited several points of inflexion, in each case occuring at the same temperatures. These inflexions represent a change in the reaction kinetics, possibly a conformational change in the enzyme. The results also indicate that the LDH 1 and2 isoenzymes are more efficient than LDH 4 and 5.

Um Lactatdehydrogenase-Isoenzyme aus Herz und Leber des Menschen bei maximaler Aktivität messen zu können, wurden die dafür erforderlichen Substratkonzentrationen (Pyruvat, NADH) und pH-Werte für die Temperaturen 25, 30, 37, 40, 45 und 50°C ermittelt. Die unter solchen Bedingungen gemessenen Geschwindigkeiten der Lactatdehydrogenase-Isoenzyme wurden nach *Arrhenius* aufgetragen (log Anfangsgeschwindigkeit gegen reziproke absolute Temperatur). Die *Arrhenius*-Plots waren für beide Isoenzyme bis 45°C linear. Zwischen 45 und 50°C scheint diese lineare Beziehung besonders für Lebergewebe nicht mehr zu gelten. Bei der Bestimmung der Aktivierungsenergie wurden für beide Isoenzympräparate mehrere Knicke, jeweils bei derselben Temperatur, beobachtet. Diese Knicke sind durch eine Änderung der Reaktionskinetik, möglicherweise der Enzymkonformation, bedingt. Die Ergebnisse sprechen außerdem dafür, daß die Isoenzyme LDH_1 und LDH_2 wirksamer sind als LDH_4 und LDH_5.

It can hardly be disputed that most clinical chemists accept the need for international agreement on standardization of the conditions under which enzyme activities are measured. Efforts to obtain such agreement have been hindered by a failure to agree upon a standard temperature. Some of the arguments in the controversy relating to whether enzyme activity should be measured at 25°C, 30°C or 37°C have been based on incomplete experimental evidence. A less than expected increase in enzyme activity between 25°C and 37°C has been used to support the argument that the enzyme has been partially denatured (or inactivated) at the higher temperature (1). The experimental evidence to support this argument was obtained by calculating the deviation of the measured activity from the activity calculated from an *Arrhenius* plot (1). Such experimental work is based on the assumption that a substrate concentration which saturates the enzyme at one temperature will saturate it at another. Activation energies obtained from enzyme reaction velocities and the *Arrhenius* equation are meaningful only if the velocities are those obtained when the enzyme is saturated with substrate (2).

The study described in this paper aimed at obtaining true *Arrhenius* relationships of lactate dehydrogenase isoenzymes derived from human heart and liver tissue by measuring the enzyme activity at 25, 30, 35, 37, 40, 45, and 50°C. To obtain these relationships it was necessary to measure enzyme activity using the pyruvate and NADH concentrations and the buffer pH which gave maximal activity with each tissue at each temperature.

Materials and Methods

Instruments

LKB 8600 Reaction Rate Analyser adjusted so that any of the temperatures stated earlier could be selected. The timing cam was also adjusted so that the timing mechanism would change at 30 second intervals.

LKB 8620 cooling stage.
Electronic instruments Ltd. 2320 pH meter.

Enzymes

Human heart and liver tissue was obtained at post-mortem examination. The tissues were washed to remove blood. Approximately 2 g of each tissue was minced and 100 ml of 67 mmol/l Sørensen phosphate buffer pH 7.2 at 0–5°C was added. The tissues were then homogenized and filtered through eight layers of cotton gauze. The filtrate was then diluted with phosphate buffer containing 50 g of albumin per litre.

Purified enzymes were not used as one aim of the study was to judge the significance of any deviation from linearity of the *Arrhenius* plots. Instability of the purified enzyme molecule may produce such deviation as may molecular changes produces during the various purification steps. Tissue homogenates had the advantage of providing a physiological buffered state in which to measure the lactate dehydrogenase activity.

Procedure

Sodium pyruvate was prepared in a broad range of reaction concentrations from 0.1 mmol/l to 8 mmol/l. Initial experiments gave the approximate optimal substrate concentration at each temperature. They were then repeated using a range of concentrations around this value. Reduced β-nicotinamide-adenine dinucleotide solutions were prepared to give reaction concentrations in the range 30 µmol/l to 240 µmol/l. Sørensen phosphate buffer, 67 mmol/l, was prepared in the pH range 6.2–8.0 at 20°C. The tissue filtrate sample volume was 20 µl and the reaction was initiated by adding 50 µl of substrate. Total reaction volume was 1.07 ml.

Results

Maximal assay conditions for lactate dehydrogenase with pyruvate as substrate were established at 25, 30, 35, 37, 40, 45 and 50°C using the heart (LDH_1, LDH_2) and liver (LDH_4, LDH_5) preparations. The initial series of experiments at each temperature investigated the effects of changes in pH over a broad range of pyruvate concentrations. The results given in figure 1 illustrate the experimental work carried out in the whole series. Maximal lactate dehydrogenase activity of the heart tissue supernatant at 50°C was found with a 3 mmol/l pyruvate concentration at pH 6.8.

The next group of experiments used the buffer and substrate concentrations which the earlier experiments had shown gave maximal activity at each temperature. The lactate dehydrogenase activity of both tissues at each temperature was then measured over a wide range of NADH concentrations. The results obtained with liver tissue at 35°C using 3.2 mmol/l pyruvate and buffer pH 7.4 are shown in figure 2. Maximal activity was obtained with 120 µmol/l NADH in the reaction mixture.

Table 1 contains full details of the conditions found to give maximal activity at each temperature with each tissue. Using these conditions the lactate dehydrogenase activity of the filtrate of freshly homogenised human heart and liver tissue was measured. These measurements for each tissue over the stated range of temperatures were carried out over five hours. This short time interval ensured that no differences found were due to loss of enzyme activity with time, or to differences in the reagents. The velocities obtained using these maximal assay conditions are presented in table 2. Using this data *Arrhenius* plots, i.e. log. initial velocity against inverse absolute temperature, were obtained from the lactate dehydrogenase activities of the heart and liver tissues (fig. 3).

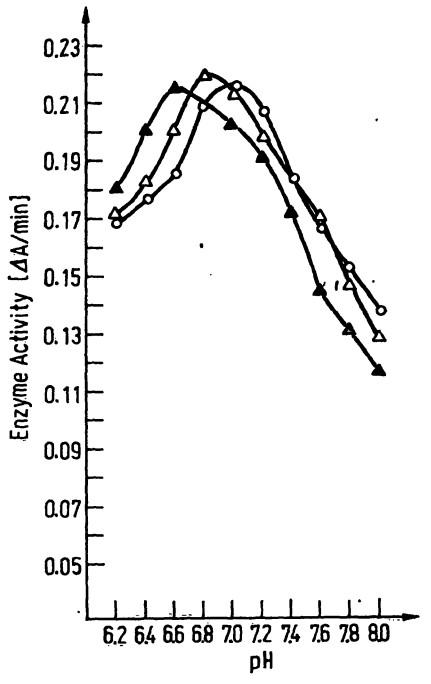

Fig. 1. Variation of enzyme activity at 50°C of heart tissue extract with buffer pH at different concentrations of pyruvate.
▲—▲ 2 mmol/l
△—△ 3 mmol/l
o—o 4 mmol/l

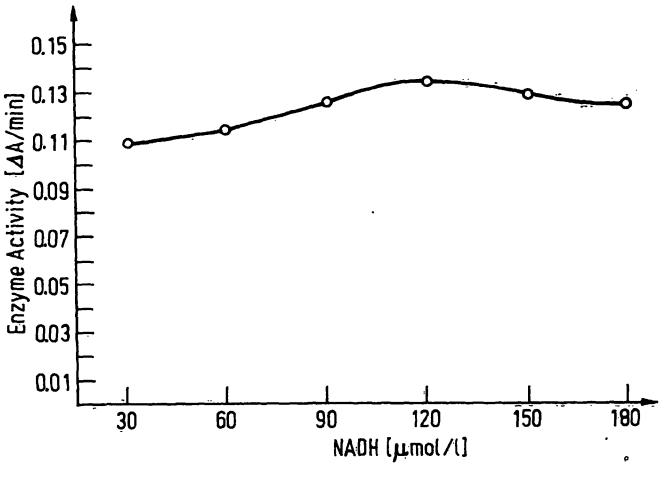

Fig. 2. Variation of enzyme activity at 35°C of liver tissue extract with NADH concentration at 3.2 mmol/l pyruvate and pH 7.4.

Tab. 1. The pyruvate concentrations, NADH concentrations and buffer pH giving maximal activity with heart and liver tissue extracts at each of the stated temperatures.

	Pyruvate [mmol/l]		Phosphate Buffer-pH		NADH[µmol/l]	
	Heart	Liver	Heart	Liver	Heart	Liver
25°C	0.3	0.7	7.2	7.2	90	120
30°C	0.4	1.7	7.2	7.4	120	120
35°C	0.6	3.2	7.2	7.4	120	120
37°C	1.0	3.7	7.2	7.4	120	120
40°C	1.5	4.2	7.0	7.4	150	150
45°C	2.3	5.1	7.0	7.4	150	150
50°C	3.0	6.2	6.8	7.2	150	150

Tab. 2. Lactate dehydrogenase activities of heart and liver tissue extracts using the maximal assay conditions at each of the stated temperatures.

Temperature	Lactate dehydrogenase activity [U/l] Liver tissue	Heart tissue
25°C	617	388
30°C	832	553
35°C	1161	819
37°C	1264	967
40°C	1626	1204
45°C	2200	1733
50°C	2632	2308

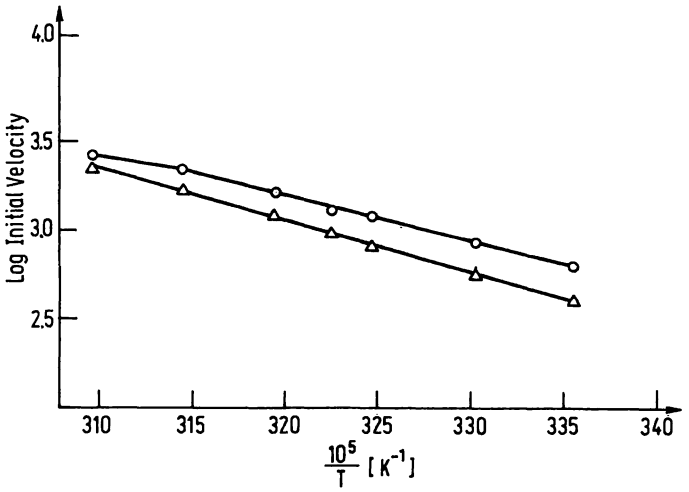

Fig. 3. Effect of temperature on the activity of the lactate dehydrogenase isoenzymes, plotted according to *Arrhenius*.
LDH_4 and LDH_5 (liver tissue) o——o
LDH_1 and LDH_2 (heart tissue) Δ——Δ

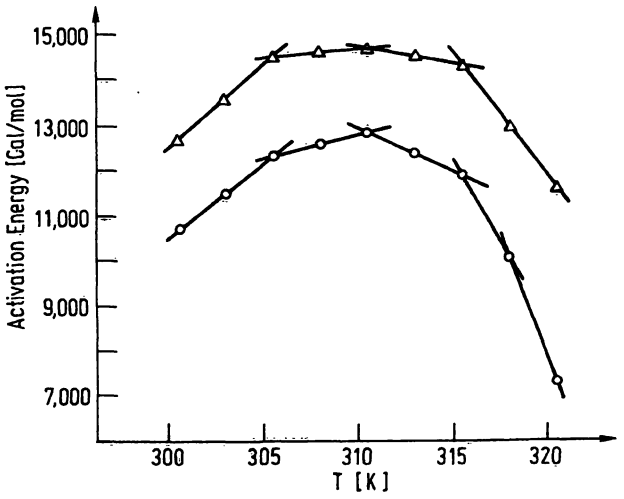

Fig. 4. Activation energies (Cal/mol) of the lactate dehydrogenase isoenzymes plotted against absolute temperature.
LDH_4 and LDH_5 (liver tissue) o——o
LDH_1 and LDH_2 (heart tissue) Δ——Δ

Activation energies (Cal/mol) were then calculated from the *Arrhenius* equation at 5°C intervals and were plotted against temperature (fig. 4).

Discussion

It has been claimed (1) that there is a significant deviation from linearity at 37°C for lactate dehydrogenase (LDH) activity of the fast and slow moving isoenzymes. However, the *Arrhenius* plots (figure 3) in this study are linear with both isoenzyme preparations up to 45°C. There is a suggestion that the linear relationship is lost between 45°C and 50°C, particularly with the liver tissue.

The activation energies found in this study were obtained from enzyme reaction velocities in their only meaningful situation, that is where the enzyme is saturated with substrate at each temperature. A similar pattern was exhibited by both the heart and liver tissue supernatants (fig. 4). This pattern was also obtained when the experiments where repeated. Interpretation of the significance of these results involves a large measure of speculation as they exhibit a number of surprising features. Both isoenzyme preparations show several points of inflexion, in each case occurring at the same temperatures. It can be safely stated from the results that the LDH_1 and LDH_2 isoenzymes are more efficient, that is they have a greater turnover number, than LDH_4 and LDH_5. Each inflexion represents a change in reaction kinetics, possibly a conformational change in the enzyme. It is interesting that both peak at 37°C (310 K) though no great significance can be read into this finding. Beyond 37°C less activation is obtained with rise in temperature.

One conclusion of this study which does not involve speculation is that no arguments produced in any controversy regarding the temperature at which enzyme activity may be measured, should be based on incomplete information. *Arrhenius* plots are not valid unless the enzyme is saturated with substrate. Conclusions should not, therefore, be drawn from them when a single concentration of substrate has been used over a range of temperatures.

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